Joint Lubricants as Delivery Methods for Multipotent Stromal Cells

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ABSTRACT

Multipotent Stromal Cells (MSC) are utilized as therapeutic agents for addressing tissue regeneration for musculoskeletal conditions, including knee osteoarthritis (OA). Currently, cell therapies lack FDA-approval for injections to alleviate joint OA. To overcome this barrier, some clinicians are utilizing autologous stem cell transplants that are not regulated. Yet, the results are mixed with a majority of patients indicating little or no relief. Major challenges in the clinical application includes poor MSC viability after isolation, extreme shear stress of the injections, maintenance of the cells in the joint capsule, and the harsh inflammatory environment of knee OA. As hyaluronic acid (HA) is an innate polymer of synovial joints that maintains cartilage viscoelastic integrity, HA-based cell delivery systems are of interest. While MSCs could be delivered in these uncrosslinked gels, it is hypothesized that they will not trap the cells in the knee joint long enough to have an effect. The aims of this study were: 1) to determine whether a commercial HA joint lubricant (Monovisc) could maintain MSC viability under different conditions, and 2) to determine the ability of cells delivered in Monovisc to reverse knee joint degeneration in an OA rat model.

INTRODUCTION

"Knee Osteoarthritis (OA) impacts 14 million U.S. citizens, with nearly 2 million people under 45 years of age and 6 million people between 45 to 64 years of age.[1] This knee joint inflammation presents as cartilage deterioration and tearing of the meniscus. Orthopedic clinicians utilize multipotent stromal cells (formerly called mesenchymal stem cells) (MSCs) or hyaluronan-based intra-articular (IA) Injections for the purposes of cartilage regeneration and meniscus repair. Autologous adipocyte MSCs, with anti-inflammatory properties, are obtained from patients in the clinic and injected directly into the joint cavity. IA Injections do not ensure target location specificity or sufficient retention of the cells. The globally approved Cartistem, first approved in Korea and manufactured by Medipost, is a regulated product combining human MSCs with an HA gel for IA utilization.

HA viscosupplements are FDA-approved products consisting of uncross-linked HA, with the ability to support cartilage elasticity within the synovial joint. Monovisc is among a commercially available HA products whose high molecular weight impacts the therapeutic duration. This product demonstrates temporary pain improvement in the affected joint with little long-term effect.

Practicing clinicians have suggested that they mix MSCs with commercially available HA products for injection hoping, without evidence, that the HA will localize cells for knee OA treatments. However, without an FDA-approved procedure for MSCs procurement in the clinic and IA administration, there is absent guidance and oversight for these procedures.[2] The FDA has launched investigations and implemented penalties for practicing outside of the parameters for MSC clinical applications.[3] Embracing the One Health initiative of sustaining animal and human health, we aim to determine whether mixing stem cells with commercial joint lubricants such as Monovisc improves OA knees outcomes compared to injections of stem cells alone. The outcomes will be used to help direct evidence-based practices in both veterinary and human health clinics.

REFERENCES

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METHODS

Rat bone marrow MSCs were cultured using standard methods. MSCs were harvested and blended with an uncross-linked HA polymer (Monovisc Intraarticular), per the CartiStem protocol of a 1:1 volume addition. Controls consisted of unencapsulated MSCs. Cell Viability data were obtained at storage conditions of 4°C, 24°C, and 37°C across the following time intervals: 30minutes, 4hours, 19 hours, 24 hours and 48 hours. Viability images stained with calcein and ethidium bromide were captured o a Biotek Cytation Multiplate Reader.

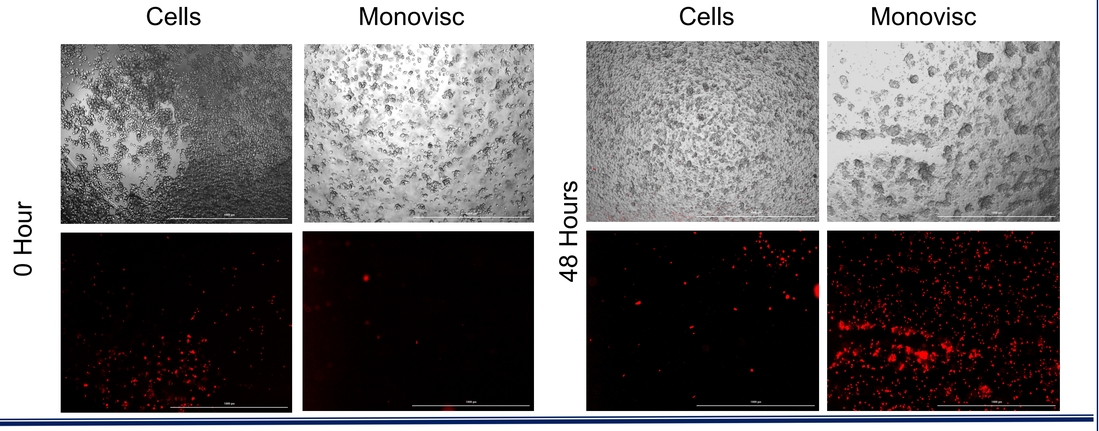
Changes in gene expression of 11 genes were conducted on cells following entrapment in Monovisc for 1 week. At the end of the week, the Monovisc was dissolved using hyaluronidase and the cells isolated and RNA extracted for qPCR.

For in vivo studies, the well-established rat knee OA model of destabilizing the medial meniscus of Sprague-Dawley rats was utilized. Destabilization of the medial meniscus resulted in overt knee OA in Sprague-Dawley rats at 4-6 weeks. A total of 18 rats had the medial meniscus destabilized in the two hind joints. The affected joints were treated with MSCs alone or MSCs delivered in Monovisc. Prior to the treatment and weekly thereafter, standard squeeze tests and calibrated pressure pain tests were administered to rats. At 6 weeks post treatment, rats were terminated and joints collected for histological processing. This study is ongoing, but knee sections will be analyzed using the Osteoarthritis Research Society International (OARSI) histopathological scoring system for interpreting osteoarthritic damage and cartilage proteoglycan, as function of treatment group effects by blinded evaluators.

RESULTS

Viability

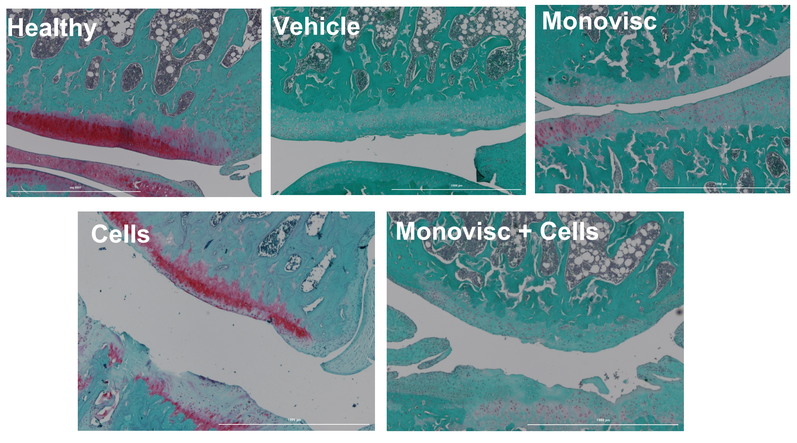
Within each storage condition, there was a greater percentage of live cells at 4 hours than at 48h hours. The greatest percentage of dead cells was found with the Monovisc + MSC blended group in the 4°C and 24°C conditions. When cultured in an incubator at 37°C, both groups had similar cell viability. The images illustrate the increased cell death noted in the Monovisc group when held at 24°C. Manual cell counts will be used in further analyzing the data. Cells = cells cultured in standard conditions. Monovisc = cells cultured in Monovisc.



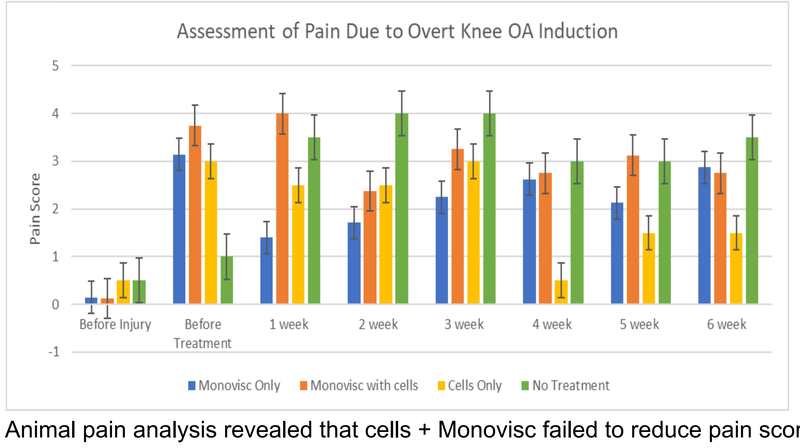
Quantity and Quality of mRNA

Sample ID	Total RNA (ug)	A260/280	A260/A230
MSCs + Monovisc	1.97	1.79	2.22
MSCs (Control)	35.78	2.01	2.24

While the starting cell counts were approximately equal, significantly less RNA could be obtained from the Monovisc group. Spectrophotometric results of the A260/280 ratio for the Monovisc group was outside of acceptable ratio for protein contamination (> 1.8), while control MSCs in all samples yielded acceptable ratio values.



Joint sections are currently being analyzed using the OARSI histopathological scoring system. However, early visual review indicates continued OA in the group of rats that received MSCs + Monovisc, while the group receiving only MSCs showed some minor improvement. The "Healthy" image is an example of healthy joint with smooth surface and high levels of proteoglycans (red) essential for cartilage health.



Animal pain analysis revealed that cells + Monovisc failed to reduce pain scores from weeks 1-6 after treatment. However, the group treated with MSCs alone had reduced pain scores at 4 weeks after treatment. In fact, the Monovisc alone and control (no treatment groups) had similar pain scores.

CONCLUSIONS

Cell viability was lower in the Cells + Monovisc group when cultured at 4°C and 24°C. In cultured at 37°C the viability at 48 hours was similar when cells were cultured alone or in Monovisc. However, only a minimal amount of RNA could be obtained from the Cells + Monovisc group after 1 week. When cells alone, cells + Monovisc, or Monovisc alone were injected into the knees of rats with OA, the only group to show a significant improvement in pain was the cells alone group at 4 weeks. This was confirmed by histological assessment at 6 weeks as the cells alone group was the only group to show an increase in proteoglycan levels indicative of healthy cartilage. Injecting the cells within Monovisc appeared to block the improvement noted with cells alone. This may be due to the fact that the cells died quickly when cultured in Monovisc. Such viscosupplements were never designed to support cell health, rather their purpose is to act as joint lubricants.